Methods

*Study Sites*

This research was conducted at the Cedar Creek Ecosystem Science Reserve (CCESR) in central Minnesota and Moores Creek Research Preserve (MCRP) in south-central Indiana. Cedar Creek is a 5,600 acre ecological field station that borders prairie and forest ecoregions with a mean annual temperature of 6.7°C and mean annual precipitation of 801 mm. Moores Creek is comprised of 261 acres of mixed deciduous hardwood forest (~ 80 years in age) with a mean annual temperature of 11.6°C and a mean annual precipitation of 1200 mm. Sites were selected within CCESR and MCRP to study the effects of vegetation type on fungal decomposition. At CCESR decomposition experiments were conducted within prairie and oak savanna sites and at MCRP litter was incubated in temperate forest stands. Within sites, vegetation communities differed in their relative abundance of AM and ECM associated plant species.

Plot or transect locations within the different vegetation types were chosen to test the effects of dominant mycorrhizal type on fungal decomposition. In prairie plots litter was decomposed beneath patches of the ECM associated herb, *Helianthemum bicknellii* and nearby patches of AM associated grass species (see Table 1). Within oak-savanna plots, a mycorrhizal gradient that was established along a 30 meter transect. Transects started in the interior of an ECM associated oak stand and then continued to the mixed ECM-AM forest-grassland edge out into the interior of an AM associated grassland. Temperate forest plots were established according to known mycorrhizal associations of dominant tree species. In all plots, trees from the dominant mycorrhizal type (AM or ECM) represented >85% of the basal area of the plot.

*Fungal Necromass Generation and Decomposition*

To capture variation in fungal tissue chemistry, we selected ectomycorrhizal fungal isolates known to differ in their melanin and N concentrations (see Table 2). Selected ectomycorrhizal species included *Cenococcum geophilum, Meliniomyces bicolor* and *Mortierella elongata*. *Meliniomyces bicolor* can form both melanized and non-melanized morphs depending on culture conditions (Fernandez and Kennedy 2018). Fungal biomass was produced in liquid cultures by inoculating 50 mL of half-strength potato dextrose broth with 3mm diameter mycelial plugs. To generate the white (non-melanized) morph of M. *bicolor*, cultures were submerged in an additional 50ml of broth, reducing the fungi’s exposure to oxygen and limiting the redox reactions that polymerize melanin precursors (Siletti *et al.* 2017: Fernandez *et al.* 2018). Following inoculation, cultures were transferred to an orbital shaker and left to shake at 80 rpm orbital for at least 30 days or until growth stopped (growing times differed among fungal isolates as melanized species tended to grow more slowly see Supplementary information).

To produce the fungal necromass, cultures were rinsed with distilled water and dried at 26°C for 24 hours. Dried fungal cultures (~25mg) were then placed into nylon mesh litter bags constructed from 53 micron mesh (Elko, Minneapolis, MN, USA). Separate litter bags were constructed for each fungal isolate and morph of *M. biclor*. During deployment, litter bags were buried at the interface between organic and mineral soil. Upon collection the necromass was removed from the litterbag and dried to a constant mass to determine mass remaining. Following mass measurements, a subset of remaining necromass was stored at -20oC for DNA analyses. While the preparation and processing of necromass was standardized across the three sites, study durations, incubation times and the individual isolates decomposed differed (see Supplementary Fig 1).

Two successive studies were conducted at the prairie site; in the first study *Cenococcum geophilum* and *Mortierella elongata* necromass was incubated for 7, 14, 21, 30 and 65 day periods beginning in July of 2017 (n = 4 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date). The second prairie study was meant to replicate the results of the first study, but with higher spatial replication. In this second study, *Cenococcum geophilum,* *Meliniomyces bicolor* (the black morph) and *Mortierella elongata* necromass was incubated for 11 and 34 days starting in late August of 2017 (n = 6 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date) . *Mortierella elongata* and *Meliniomyces bicolor* (both the white and black morphs) necromass was incubated in the oak-savanna site for 14, 28, 42 and 56 day increments starting in (June or July) of 2017 (n = 3 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date. *Mortierella elongata* and *Meliniomyces bicolor* (the black morph) were decomposed at the forest site over 14 day, 31 and 92 days beginning in late July of 2017 (n = 7 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date).

Soil moisture was collected at the same time litter bags were harvested. pH measurements and surrounding soil. Soil was sieved (2mm sieve) or roots/ rocks were removed prior to measurements being made

*Identification of Bacterial and Fungal Decomposer Communities*

For a comparison of microbial decomposer communities among sites we performed molecular analyses for the black morph of *Meliniomyces bicolor* and *Mortierella elongata* at the oak savanna and temperate forest sites. Microbial genomic DNA was isolated from soil and necromass samples using DNeasy PowerSoil Extraction Kits (QIAGEN, Germantown, MD, USA). DNA extractions were done according to the manufacturer’s instructions with the addition of a bead-beating step prior to the start of the extraction to better homogenize necromass samples (Fernandez and Kennedy 2018). Positive and negative controls were included for both bacteria and fungi. Positive controls included mock communities from the Human Microbiome Project for bacteria and for fungi, the “SynMock” synthetic mock community developed by Palmer *et al.* (2017). We also extracted DNA from necromass that had not been incubated in litterbags. Negative controls included lysis tubes lacking substrate and PCR reactions with no DNA template added

Bacteria and fungi colonizing necromass samples and nearby soil were identified using high-throughput sequencing. For fungal identification we used the ITS2 primer pair designed by Taylor *et al.* (2016). For bacteria, we used 515F-806R primers to target the V4 region of the 16S rRNA gene. PCR reaction reagents and cycling conditions followed the Illumina MiSeq two-step PCR amplicon sequencing protocol. If initial PCRs were not successful, we performed dilutions or increased cycle numbers. Following amplification PCR products were visualized by gel electrophoresis and cleaned using the Charm Just-a-Plate Purifiation and Normalization Kit (Charm Biotech, San Diego, CA, USA). Successful bacterial and fungal amplicons for each sample were pooled at equimolar concentration and sequenced together on a full MiSeq lane (2 x 300 bp V3 Illumina chemistry) at the University of Minnesota Genomic Center.

To process bacterial and fungal HTS amplicon sequences we used the AMPtk pipeline (amplicon toolkit; Palmer *et al.* 2017) v1.1 primarily utilizing the default settings (<https://amptk.readthedocs.io/en/latest/index.html>). In summary, paired end reads were merged using VSEARCH (check this) and then subjected to quality trimming. Following pre-processing the UNOISE3 OTUs were clustered using the denoising algorithm run in USEARCH10. We applied a 0.0005 abundance cut-off to the bacterial data to eliminate very low abundance OTUs that were thought to be spurious. For the fungal data, SynMock abundances were used to determine the filtering threshold. For Fungi, functional assignments (i.e., saprotrophic, symbiotrophic, and pathotrophic trophic guilds) were made with FUNGuild (Nguyen *et al*. 2016). We further parsed symbiotrophic fungi into ectomycorrhizal and arbuscular mycorrhizal guilds. Finally, when possible, we manually assigned guilds to OTUs that were unassigned (due to missing genus taxonomy) as well as those OTUs that were assigned multiple trophic modes.

*Statistical Analyses*

To test differences in soil moisture and pH among sites we performed an ANOVA.

A three-way full factorial linear mixed model was con- structed to test the fixed effects of incubation time, melanization and site on log-transformed mass remaining data. Block was also included as the random effect.

You neglect dependencies among observations – individuals from the same block are not independent, yielding residuals that correlate within block.

A linear mixed effects model was used to

Where X is the mass remaining at time t and k is the decomposition rate, calculated as the remaining mass at time (t) divided by the initial mass for each litterbag.

Results

3 vegetation types

Within each vegetation type there is a mycorrhizal treatment which

Variation due to the different experiments

We describe such situations as having partially crossed grouping factors for the random effects.

Completely crossed each isolate in each vegetation type for each sampling date

At this point we will fit models that have random effects for isolate, incubation time, and experiemnt (or the dept:service combination) to these data. In the next ch

explicitly in variation among and by groups. This is where a mixed-effect modeling framework is useful

standing of how to explain the relationships among the fixed and random effects in terms of the levels of the hierarchy.

Hello,

I have a dataset which combines 3 experiments measuring the decomposition of fungi. The experiments were conducted at three different sites, differing their dominant vegetation (i.e., prairie, oak savanna and forest). The species of fungi decomposed and the times the fungi were decomposed differ among the 3 experiments/sites. There are two species of fungi which were included at all the sites, but not all combinations of factors occur (thus my design is partially crossed). I have decided to use a mixed linear effects model to deal with any variation caused by differences among experiments-treating the partially crossed grouping factors as a random effect.

I perform my statistical analyses in R and I am looking for someone to help me confirm that I am using the correct lmer () model syntax to specify relationships among fixed and random effects.